Metabolism of 2-Chloro-1-(2,4,5-trichlorophenyl)vinyl Dimethyl Phosphate in the Dog and Rat

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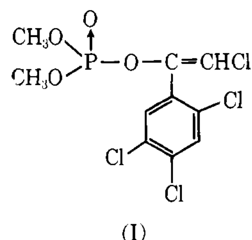
A single oral dose of 2-chloro-1-(2,4,5-trichlorophenyl)-[^{14}C]vinyl dimethyl phosphate to rats is almost completely eliminated in 4 days. Seventy-eight per cent of the ^{14}C is excreted in the urine, 16.5% in the feces, and 0.5% in the expired gases; 0.8% of the ^{14}C is present in the gut and contents after 4 days. After oral administration of the compound to dogs, 92% of the ^{14}C is excreted in the urine and feces during 4 days. The compound is completely metabolized in dogs and rats; unchanged insecticide is absent from the urine. The metabolic products in the urine of rats and dogs were as follows (per cent of total dose in rat and dog, respectively, given in parentheses): 2,4,5-trichlorophenylethanediol glucuronide (8, 12%), [1-(2,4,5-trichlorophenyl)ethyl-β-D-glucopyranosid]uronic acid (35, 0%), 2,4,5-trichloromandelic acid (24, 12%), 2-chloro-1-(2,-4,5-trichlorophenyl)vinyl methyl hydrogen phosphate (4, 46%), 2,4,5-trichlorophenylethanediol (2.5, 4%), and 1-(2,4,5-trichlorophenyl)ethanol (2, 0%).

2 - Chloro - 1 - (2,4,5 - trichlorophenyl)vinyl dimethyl phosphate (Phillips *et al.*, 1963) (I) (SD 8447) is a member of the vinyl phosphate series of insecticides which possesses a very favorable mammal-to-insect toxicity ratio. The potential use of this compound as an insecticide makes a knowledge of its metabolism and excretion in mammals desirable. The results of this study also supplement knowledge (Hutson *et al.*, 1967) of the metabolic fate of this type of molecule. The use of the compound labeled with ^{14}C at both vinyl carbon atoms has made possible a thorough study of the metabolic fate of the chlorophenyl vinyl part of the molecule. The synthesis, insecticidal activity, and preliminary metabolism studies have recently been reported by Whetstone *et al.* (1966).



(I)

Materials

All melting points are uncorrected, and all reagents were AnalaR grade.

2 - Chloro - 1 - (2,4,5 - trichlorophenyl)vinyl Dimethyl Phosphate (SD 8447). SD 8447, synthesized by the reaction of trimethylphosphite with 2,4,5-trichlorophenacylidene dichloride (Gilbert *et al.*, 1961), was obtained from Shell Development Co., New York, and recrystallized from hot hexane to yield the pure beta isomer, m.p. 97–98° C. (The beta isomer is defined as the geometrical isomer in which the chlorine atom on carbon 2 of the vinyl group and the chlorophenyl substituent on carbon 1 are in the trans orientation.)

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2-Chloro-1-(2,4,5-trichlorophenyl)-[^{14}C]vinyl Dimethyl Phosphate ([^{14}C]SD 8447). The Shell Development Co., Modesto, Calif., synthesized the radioactive compound which was made by the route described for the unlabeled compound but using 2,4,5-trichlorophen-[U-^{14}C]acylidene dichloride. The labeled compound (2.46 μc./mg.) was found by thin-layer chromatography on Kieselgel G developed with hexane–acetone (4 to 1, v./v.) to be 99.5% pure.

2-Chloro-1-(2,4,5-trichlorophenyl)vinyl Methyl Hydrogen Phosphate. SD 8447 (10 grams) was refluxed in 50 ml. of 30% (by volume) H_2SO_4 for 1 hour, and the solution was extracted with dichloromethane. The product was extracted from the dichloromethane with 2% aqueous potassium carbonate solution leaving unchanged compound and ketones in the organic phase. The product was re-extracted from the acidified aqueous solution with ether and crystallized from dichloromethane to yield 2.5 grams of 2-chloro-1-(2,4,5-trichlorophenyl)vinyl methyl hydrogen phosphate, m.p. 137–138° C. Found, C, 30.7; H, 2.1; Cl, 40.4; P, 9.0. $C_9H_7Cl_4P$ theory: C, 30.7; H, 2.0; Cl, 40.3; P, 8.8%.

2,4,5-Trichloromandelic Acid. Five grams of 2,4,5-trichlorophenacylidene dichloride was treated with 25 ml. of 10% (w./v.) sodium hydroxide solution for 30 minutes at room temperature. The solution was then acidified with 5*N* hydrochloric acid to pH 1 to 2 and extracted with ether. The dried ether extract was evaporated and the product recrystallized from benzene to yield 2 grams of 2,4,5-trichloromandelic acid, m.p. 120–133° C. Found: C, 37.5; H, 1.8; Cl, 41.4. $C_8H_5O_3Cl_3$ theory: C, 37.6; H, 2.0; Cl, 41.7%. The N.M.R. spectrum agreed with the structure of the compound, but the melting point could not be improved by further recrystallization.

1-(2,4,5-Trichlorophenyl)ethanol. Trimethyl phosphite (15 grams) was added with shaking to a solution of 26 grams of 2,4,5-trichlorophenacyl chloride in 150 ml. of ether. The mixture was then refluxed for 24 hours, when an additional 10 grams of trimethyl phosphite was added, and the refluxing continued for 16 hours. The solution was then evaporated to an oil which was shown by thin-layer chromatography on

Kieselgel G (hexane–acetone, 4 to 1, v./v.) to consist largely of one component, presumably 1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate (Perkow *et al.*, 1952). The oil was refluxed with 200 ml. of 30% (by volume) aqueous H_2SO_4 for 8 hours. The cooled solution was extracted with ether, dried, and evaporated to yield 26.8 grams of crude product which was recrystallized twice from petroleum ether (b.p. 40-60°) to yield 2,4,5-trichlorophenylmethyl ketone, m.p. 45° C. Found: C, 43.2; H, 1.9; Cl, 47.4. $C_8H_5OCl_3$ theory: C, 43.0; H, 2.25; Cl, 47.6%. This product (20 grams) was dissolved in 200 ml. of methanol, and 5 grams of sodium borohydride was added in portions with cooling. After standing for 30 minutes, the solution was evaporated, diluted with water, neutralized with H_2SO_4, and extracted with ether. The dried ether extract was evaporated and the product recrystallized from petroleum ether (b.p. 40–60°) to yield 18 grams of 1-(2,4,5-trichlorophenyl)ethanol, m.p. 88–89° C. Found: C, 42.9; H, 3.1; Cl, 46.9. $C_8H_7OCl_3$ theory: C, 42.6; H, 3.1; Cl, 47.2%.

2,4,5-Trichlorophenylethanediol. Ten grams of 2,4,5-trichlorophenacyl chloride was dissolved in 200 ml. of acetone and 20 grams of dry powdered potassium benzoate was added. The mixture was refluxed for 48 hours, evaporated to dryness, and partitioned between water and ether. The ether-soluble product was decolorized using animal charcoal and ethanol and was crystallized four times from acetone–hexane to yield 2 grams of 2,4,5-trichlorophenacyl benzoate m.p. 114-5° C. Found: C, 52.0; H, 3.1; Cl, 30.8. $C_{15}H_{10}O_3Cl_3$ theory: C, 52.3; H, 2.9; Cl, 30.9%. This compound (500 mg.) was reduced with sodium borohydride in methanol and the solution was diluted with 5*N* HCl and extracted with ether. The product was dissolved in 10 ml. of methanol, 1 ml. of 1*N* sodium methoxide in methanol was added, and the solution was warmed to 60° C. for 10 minutes. The product was isolated by ether–water partition of the evaporated solution and recrystallized from ether–hexane to yield 200 mg. of 2,4,5-trichlorophenylethanediol m.p. 90–91° C. Found: C, 40.0; H, 3.0; Cl, 44.1. $C_8H_7O_2Cl_3$ theory: C, 39.8; H, 2.9; Cl, 44.0%.

2,4,5-Trichlorophenacylidene chloride (b.p. 103–105° C./1.15 mm.), 2,4,5-trichlorophenacyl chloride (m.p. 66-67° C.), and 2,4,5-trichlorobenzoic acid (m.p. 166° C.) were supplied by Shell Grundlagenforschung Gesellschaft m.b.H., Schloss Birlinghoven, bei Siegburg, Germany.

Bovine Liver β-Glucuronidase. This enzyme (1 gram, 560,000 units) was obtained from the Sigma Chemical Co., St. Louis, Mo.

Methods

Experiments with Animals. Young adult rats (approximately 2 months old, 150–200 grams body weight), Porton strain, maintained as a specific pathogen-free colony in this laboratory, were used. 2-Chloro-1-(2,4,5-trichlorophenyl)-[^{14}C]vinyl dimethyl phosphate ([^{14}C]SD 8447) (2.46 μc./mg., 3.3 mg.) was administered in 1 ml. of olive oil by stomach tube to each of six male and six female rats. The animals were kept in all-glass metabolism cages under the conditions described by Wright *et al.* (1965). Unrestricted food and water were supplied, the urine and feces were collected daily and stored at −20° C., and the exhaled gases were monitored for $^{14}CO_2$. The rats were killed after 4 days, and the alimentary canal, skin plus hair, and the remaining carcass were separately assayed for radioactivity.

In a further experiment, one male rat (body weight 400 grams) was dosed by stomach tube with 1 ml. of olive oil containing 1.6 mg. (3.92 μc.) of [^{14}C]SD 8447. After 20 minutes, a biliary fistula was established, and the animal was then placed in a restraining cage with access to food and water. Bile was collected for a 24-hour period.

Adult beagle hounds (7–14 kg. body weight), maintained for 4 to 5 years as a closed colony in the laboratory, were used for metabolism studies in the dog. Two animals of each sex swallowed a gelatine capsule containing 3.3 mg. (2.46 μc./mg.) of [^{14}C]SD 8447 in 1 ml. of olive oil. The animals were kept in stainless steel metabolism cages as described by Wright *et al.* (1965), the usual diet was supplied, and urine and feces were collected daily for 4 days.

Extraction of Radioactive Material from the Urine and Bile of Treated Animals. Days 1 and 2 rat urine samples, after assay of radioactivity, were bulked and adjusted to pH 1.9 with aqueous HCl. Continuous extraction with ether in a Schacherl apparatus for 18 hours gave 95% extraction of the radioactive material from the aqueous phase. Dog urine treated similarly gave 90% of the ^{14}C in the ether phase. Bile (17 ml.) was diluted to 100 ml. with water, acidified as above, and extracted twice by shaking with 100-ml. portions of ether This procedure extracted 75% of the ^{14}C from the bile; the remaining 25% could not be extracted with ether, and was not examined further.

Chromatography. Metabolites in the solvent extracts of urine from treated animals were separated in 18 hours by descending paper chromatography on Whatman No. 1 filter paper sheets using *n*-butanol–2*N* NH_4OH (1 to 1, v./v., top phase) as the developing solvent The dried papers were scanned for radioactivity. Phenoxyethanol–silver nitrate (Mitchell, 1958) was used to detect chlorine-containing compounds. Thin-layer chromatography was carried out on Kieselgel G (E. Merck A-G, Darmstadt, Germany). Carbon-14–labeled metabolites were detected by scanning. For identification by cochromatography, ^{14}C-labeled material and synthetic compounds were always applied to the same origin to obviate the possibility of error from variable R_f values. 2,4,5-Trichlorophenylethanediol and 1-(2,4,5-trichlorophenyl)ethanol were analyzed using benzene–methanol (96 to 4, v./v.) (R_f, 0.1 and 0.55, respectively) and hexane–ether (9 to 1, v./v.) (R_f, 0 and 0.25) as solvents.

Measurement of Radioactivity. Measurement of radioactivity in urine, feces, gut, skin plus hair, and remaining carcass, and of $^{14}CO_2$ in the expired gases were made by the methods described by Wright *et al.* (1965). A Tri-Carb spectrometer (Packard Instrument Co., La Grange, Ill.) was used for liquid scintilla-

tion counting. Zones of radioactivity on paper chromatograms were scanned with a Nuclear-Chicago Actigraph II equipped with a gas-flow cell utilizing a helium–*n*-butane (98.5 to 1.5, v./v.) mixture. Thin-layer plates were scanned with a gas-flow cell utilizing an argon–methane (9 to 1, v./v.) mixture.

Enzymic Hydrolysis of Metabolites. Carbon-14–labeled metabolites were dissolved in 2 ml. of 0.2*M* sodium acetate, pH 5.0, and incubated with β-glucuronidase (1000 units) at 37° C. for 18 hours. Urine was adjusted to pH 5.0 with dilute H_2SO_4 and β-glucuronidase (500 units/ml.) added. After incubation, neutral products were extracted directly from the digest with ether; unhydrolyzed polar starting materials were recovered by extraction with ether after acidification of the digest. Appropriate control digests were carried out.

Confirmatory Isotope Dilution Analysis. A known weight of synthetic carrier compound was added to a volume of urine of known ^{14}C content, to a portion of ether extract of urine, or to a solution of chromatographically purified metabolite. The solution in methanol or water was allowed to stand overnight, if necessary in the presence of β-glucuronidase, and the carrier compound was recovered by crystallization and recrystallization until three consecutive samples possessed constant specific activity. The solvents used for recrystallization were those described in the materials section for each compound.

Results

Excretion and Retention of Radioactive Material in Animals Dosed with 2-Chloro-1-(2,4,5-trichlorophenyl)-[^{14}C]vinyl Dimethyl Phosphate. When six male and six female rats were each treated orally with a single dose of the labeled compound, the radioactive material was largely eliminated from the body via the kidneys. Most of the remainder of the ^{14}C appeared in the feces; a small amount, 0.5% of the dose, appeared in the expired gases, presumably as $^{14}CO_2$. The dose was quantitatively eliminated from rats in 4 days, 95% (85 to 102%) being the proportion of the dose found in urine, feces, and gut. Traces (0.5%) were found in skin plus hair, and in the remaining carcass. Since there was no significant sex difference in the pattern of elimination of ^{14}C, the sexes were not treated separately (Tables I and II), and the days 1 and 2 urine samples from the animals were bulked after assay of radioactivity.

The daily rates of excretion by various routes are shown in Table II. The excretion of radioactive material by the kidneys was rapid—i.e., 44 to 78% of the dose appeared during 0 to 24 hours and 4 to 15% during 24 to 48 hours. The excretion of 6% (0 to 25%)

Table I. Excretion and Retention of Radioactivity in Rats after Oral Administration of 2-Chloro-1-(2,4,5-trichlorophenyl)-[^{14}C]vinyl Dimethyl Phosphate

	Recovery of ^{14}C (% of Dose) after 4 Days						
	Urine	Feces	Expired gases	Gut and contents	Skin and hair	Remaining carcass	Total
Mean	78.0	16.5	0.5	0.8	0.5	0.5	96.4
Range	51.1–91.4	1.6–43.0	0.4–0.7	0.2–3.2	0.3–1.0	0.3–0.7	86.2–102.8
No. of independent observations[a]	11	11	5	11	11	11	

[a] One animal died by asphyxiation during the first 24 hours.

Table II. Rates of Urinary and Fecal Elimination of Radioactivity in Rats and Dogs after Oral Administration of 2-Chloro-1-(2,4,5-trichlorophenyl)-[^{14}C]vinyl Dimethyl Phosphate

Animal No.	^{14}C Elimination (% of Dose)							
	0–24 Hr.		24–48 Hr.		48–72 Hr.		72–96 Hr.	
	Urine	Feces	Urine	Feces	Urine	Feces	Urine	Feces
Rat 1	69.2	9.4	7.4	2.2	5.1	1.8	0.7	1.1
2	68.0	12.5	5.9	8.0	1.1	1.6	0.5	0.3
3	44.0	25.1	3.7	7.7	2.5	9.3	0.9	0.9
4	55.5	0	5.0	3.4	8.9	2.1	4.9	2.3
5	78.1	2.6	7.5	3.5	2.4	1.3	0.2	0.5
6	59.5	7.2	8.8	5.8	2.8	1.6	5.4	1.5
7	75.1	6.6	9.1	1.1	2.0	0.2	0.9	0.5
8	45.9	0.1	8.0	27.6	3.1	11.3	1.6	3.4
9	70.2	5.8	8.2	6.2	1.4	0.7	1.6	0.1
10	73.1	0.3	14.7	0.2	2.5	0.4	1.2	0.7
11	75.8	1.2	11.4	1.3	1.6	1.2	1.1	0.6
Dog 1	74.7	9.6	3.5	1.2	1.5	0.6	0.5	No feces
2	75.2	13.8	2.7	1.5	0.6	0.3	0.2	0
3	74.7	7.5	3.9	1.5	0.6	0.3	0.2	0
4	84.8	3.9	3.2	0.3	1.0	0.3	0.4	0

in the first 24-hour fecal sample suggests that absorption from the gut may have been incomplete in some of the animals—e.g., rat No. 3.

A male rat, in which a biliary fistula had been established immediately after dosing, excreted 40% of the dose in the 0- to 24-hour bile sample. This result, however, cannot be correlated with those of the excretion-retention study because the animal was not in a normal condition.

The very low yield of $^{14}CO_2$ (0.5%) suggests that the ^{14}C-labeled vinyl group and the two-carbon side chain in the metabolites remain intact.

Dogs treated orally with the labeled insecticide excreted 77% (75 to 85%) of the dose via the kidneys and 9% (4 to 14%) via the fecal route during 0–24 hours. The daily excretion data are shown in Table II. No sex difference in the routes or rates of elimination was apparent. A comparison of the radioactivity in the pooled 1- and 2-day urine samples from rat and from dog (Table II) shows that dogs excrete ^{14}C via the kidneys at a slightly higher rate than rats.

Identification of ^{14}C-Labeled Metabolites in the Urine of Rats and Dogs Dosed with 2-Chloro-1-(2,4,5-trichlorophenyl)-[^{14}C]vinyl Dimethyl Phosphate. Continuous ether extraction for 18 hours at pH 1.9 of urine from treated animals gave 95% and 90% of the ^{14}C from rat and dog urine, respectively. The ether extracts were concentrated and analyzed by paper chromatography in *n*-butanol saturated with 2*N* NH_4OH. Scanning of the chromatograms for radioactivity revealed six zones in rat urine and five zones in dog urine, four of which corresponded to those in the rat (Table III). R_f values and relative amounts of radioactivity in the zones are shown in Table III.

Zone *G* (rat) possessed the same R_f value as the insecticide and its hydrolysis product, 2,4,5-trichlorophenacyl chloride. This zone was eluted from 3 MM paper chromatograms and analyzed by thin-layer chromatography (benzene–methanol, 96 to 4, v./v.). Radioactive zone *G* was resolved into two components with R_f values 0.1 (70%) and 0.5 (30%) which were clearly different from unchanged SD 8447 (0.3) and 2,4,5-trichlorophenacyl chloride (0.6). The two components were later shown to be the aglycones of two glucuronide metabolites (A and B). Thus, neither the unchanged insecticide nor its hydrolysis product, the phenacyl chloride, was excreted via the kidneys.

The metabolism of a related vinyl phosphate and a chlorophenacyl chloride (Hutson *et al.*, 1967) served as a guide for the synthesis of possible metabolites which were then compared with the radioactive urine extracts by paper chromatography (Table III). Chromatographic data and confirmatory isotope dilution analyses (for details see methods section and Table IV) revealed that zones *E* and *F* in both rat and dog urine extracts consisted of 2,4,5-trichloromandelic acid and 2-chloro-1-(2,4,5-trichlorophenyl)vinyl methyl hydrogen phosphate, respectively. Scanning of radiochromatograms and assay by isotope dilution analysis gave results in fair agreement. Zones *A* and *B* were isolated from rat urine extract by chromatography on 3 MM paper. Hydrolysis of zone *B* material in 2*N* H_2SO_4 for 3 hours resulted in complete conversion to a fast-moving spot on paper chromatograms (R_f 0.9). Accordingly, zones *A* and *B* were incubated with β-glucuronidase and completely hydrolyzed to 2,4,5-trichlorophenylethanediol and 1-(2,4,5-trichlorophenyl)-ethanol which were identified by comparison with synthetic materials by thin-layer chromatography in two solvents (see methods section). The identity of the enzymically produced material from zone *B* (rat) was confirmed by isotope dilution on the purified aglycone (Table IV). The identity of the material in zone *A* (dog) was confirmed by isotope dilution with authentic 2,4,5-trichlorophenylethanediol of the original specimen of dog urine after treatment with β-glucuronidase. There is no evidence, however, to distinguish which of the two hydroxyl groups of the diol is involved in conjugation with glucuronic acid.

Thus, among the products excreted in the urine of rats and dogs treated with 2-chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate, the *O*-demethyl compound accounts for 5.6% of the dose in rats and 42.9% in dogs. [1-(2,4,5-Trichlorophenyl)ethyl-β-D-glucopyranosid]uronic acid accounts for about 45% of the dose in rats and is absent from the urine of treated dogs. 2,4,5-Trichloromandelic acid represents 24.3% and 15% of the dose in rats and dogs, respectively,

Table III. ^{14}C-Labeled Metabolites of 2-Chloro-1-(2,4,5-trichlorophenyl)-[^{14}C]vinyl Dimethyl Phosphate in the Ether Extracts of Urine of Treated Rats and Dogs

Zone	R_f	Relative Amounts of ^{14}C in Each Zone: Rat extract	Dog extract	Action of β-Glucuronidase: Rat	Dog	Identity (Based on Chromatography and Isotope Dilution Analysis)
A	0.25	10	15	+	+	2,4,5-Trichlorophenylethanediol glucuronide
B	0.3	45	–	+		[1-(2,4,5-Trichlorophenyl)ethyl-β-D-glucopyranosid]uronic acid
C	0.4	5	–			
D	0.5	–	5			
E	0.6	30	15	–	–	2,4,5-Trichloromandelic acid
F	0.7	5	60			2-Chloro-1-(2,4,5-trichlorophenyl)vinyl methyl hydrogen phosphate
G	0.9	5	5			Aglycones of glucuronides above

Table IV. Isotope Dilution Analysis of Various Urine Samples and Extracts
(For details see methods section)

Carrier Compound	Animal	Source of ^{14}C Analyzed	^{14}C in Sample, Counts/Sec.	Wt. of Carrier Added, Mg.	No. of Recrystallizations	Specific Activity of Isolated Carrier, Counts/Sec./Mg.	Amount of ^{14}C Metabolite in Sample, % of Total Radioactivity
2-Chloro-1-(2,4,5-trichlorophenyl)-vinyl methyl hydrogen phosphate	Rat	Urine	69500	811.0	8	24.2	5.65
	Dog	Urine	51180	532.0	4	47.4	49.2
2,4,5-Trichloromandelic acid	Rat	Urine	137883	593.4	5	56.5	24.3
	Dog	Urine	51180	709.4	5	10.8	15.0
1-(2,4,5-Trichlorophenyl)ethanol	Rat	Zone *B*[a]	37550	614.2	4	54.7	89.6[a]
2,4,5-Trichlorophenylethanediol	Dog	Urine	50526	113.4	3	55.05	12.3

[a] Isolated by paper chromatography and hydrolyzed with β-glucuronidase. Result indicates the fraction of the ^{14}C in zone *B*.

and 2,4,5-trichlorophenylethanediol glucuronide, for 10% and 12.3%, respectively. About 5% of the ^{14}C in ether extracts of rat urine consists of a mixture of 2,4,5-trichlorophenylethanediol and 1-(2,4,5-trichlorophenyl)ethanol, the aglycones derived from the two glucuronide conjugates. 2,4,5-Trichlorophenylethanediol (5%) also occurs in the urine of treated dogs. These alcohols may have been excreted as such or may have been produced by hydrolysis of glucuronide conjugates in the urine or during the work-up processes. Thus, rats and dogs exhibit a considerable difference in the relative proportions of the various metabolites found in the urine.

The 0- to 24-hour bile from one rat, treated orally with 2-chloro-1-(2,4,5-trichlorophenyl)-[^{14}C]vinyl dimethyl phosphate contained 40% of the ^{14}C dose. On extraction with ether and analysis by paper chromatography, the radioactivity appeared as one component (R_f 0.35) which cochromatographed with zone *B* (rat) metabolite. The ether extract was treated with β-glucuronidase and thus converted to 1-(2,4,5-trichlorophenyl)ethanol, identified by thin-layer chromatography in two solvents with authentic standard material. Thus, the ^{14}C-labeled metabolite in the bile is identical to zone *B* of rat urine—i.e., [1-(2,4,5-trichlorophenyl)ethyl-β-D-glucopyranosid]uronic acid.

Discussion

2-Chloro-1-(2,4,5-trichlorophenyl)vinyl dimethyl phosphate (SD 8447) is rapidly metabolized and excreted by rats and dogs after oral ingestion. Unchanged SD 8447, which would appear in zone *G* (Table III), is not excreted in the urine of either species. The excretion of metabolites in the urine is slightly more rapid in dogs than in rats, and gross differences in the amounts of the various metabolites are apparent. The rapid

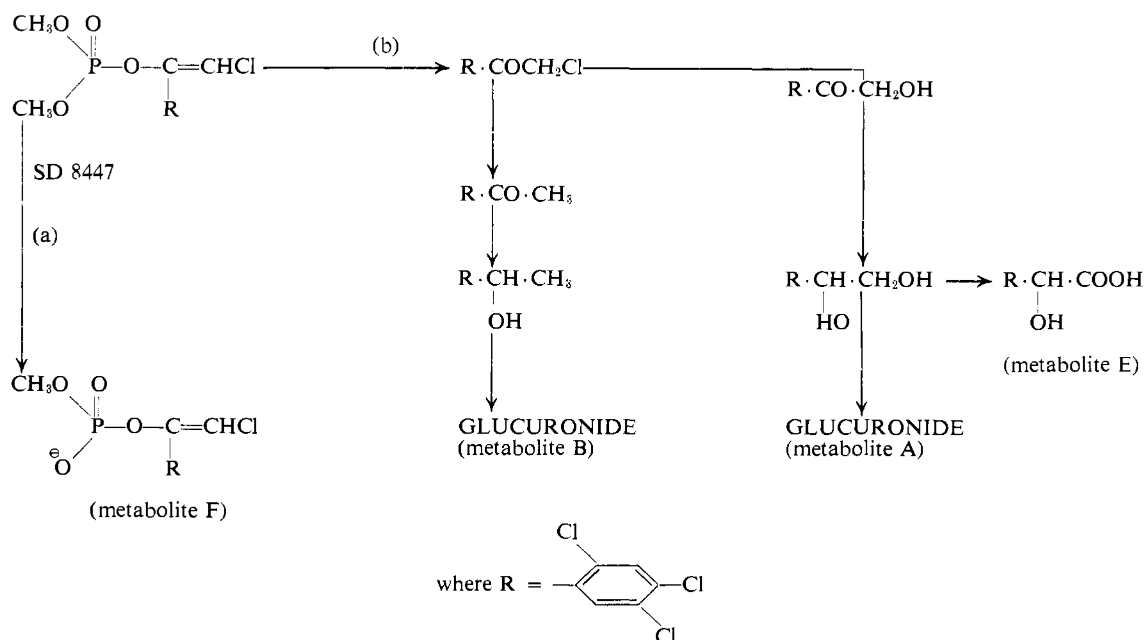
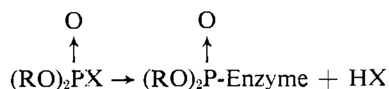


Figure 1. Possible routes of metabolism of SD 8447 in animals

rate of excretion of ^{14}C by the dog is probably related to the high yield (50 to 60%) of the strongly acidic 2-chloro-1-(2,4,5-trichlorophenyl)vinyl methyl phosphate ion (zone *F*) which appears to be rapidly cleared by the kidneys. The rat excretes only 5.6% of the ^{14}C dose as this material, the major product being [1 - (2,4,5 - trichlorophenyl)ethyl - β - D-glucopyranosid]-uronic acid (zone *B*). 2,4,-Dichlorophenacyl chloride is metabolized in the rat to the corresponding chlorophenylethyl glucuronide and the chloromandelic acid (Hutson *et al.*, 1967), thus the zone *B* glucuronide probably arises from 2,4,5-trichlorophenacyl chloride via the route shown in Figure 1. The very large difference in yields in the vinyl methyl hydrogen phosphate (zone *F*) and the glucuronide (zone *B*) in the two species (the latter is not excreted by the dog) suggests that two different pathways of detoxification may be operative in the two species. The dog may possess an efficient system for the removal of an alkyl or alkoxy group from the phosphate triester (route *a*, Figure 1). Different ratios of metabolic products in the rat may be a consequence of detoxification via P—O-vinyl bond fission (route *b*, Figure 1) by an A- or B-type esterase (Aldridge, 1953). B esterases are inhibited by organophosphates (Heath, 1961), the reaction being of the type,



$$(RO)_2\overset{O}{\overset{\uparrow}{P}}X \rightarrow (RO)_2\overset{O}{\overset{\uparrow}{P}}\text{-Enzyme} + HX$$

In this case, HX would be the enol form of 2,4,5-trichlorophenacyl chloride which would be metabolized further to the observed products (Hutson *et al.*, 1967). When the ^{32}P-labeled insecticide is administered to rats (Whetstone *et al.*, 1966), radioactive material is excreted in the urine at a rate similar to that found in the present study; however, fecal elimination of ^{32}P is slower than that of ^{14}C—e.g., 6% of the dose is found in the combined days 5, 6, and 7 fecal samples and 9 to 16% of the radioactivity is then found in the carcass. This may be due to ^{32}P being retained in the normal metabolism of the animal; on the other hand, B-esterase inhibition as a mode of detoxification may give a difference in rate of excretion of vinyl-^{14}C and ^{32}P. The former is liberated in a simple organic molecule while the ^{32}P would remain attached to the esterase protein. The major site of detoxification in the rat is probably the liver since the sole ^{14}C-labeled metabolite excreted in the bile of one animal studied is one of the terminal glucuronide metabolites. The site and the mode of detoxification of the vinyl phosphates is under further investigation.

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